25th International Symposium of the International Scientific Centre for Fertilizers "Significance of Sulfur in High-Input Cropping Systems", Groningen (The Netherlands), September 5-9, 2017

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Luit J. De Kok[2], Silvia Haneklaus[1], Ewald Schnug[1]

25th International Symposium of the Scientific Centre for Fertilizers "Significance of Sulfur in High-Input Cropping Systems"
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[1]Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen
Institut für Pflanzenbau und Bodenkunde
[2]University of Groningen
Faculty of Science and Engineering

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Kontaktadresse/ Contact
Dr. Silvia Haneklaus
Federal Research Centre for Cultivated Plants
Institute for Crop and Soil Science
Bundesallee 50
38116 Braunschweig
Germany

Telefon +49 (0) 531 596 2121
Telefax +49 (0) 531 596 2199

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Preface

Founded in 1933, CIEC (Centre International Engrais Chimique) has accomplished to be one of the oldest scientific organizations that foster the implementation of scientific knowledge in the field of fertilizer research into crop production. The 25th International CIEC Symposium in Groningen (The Netherlands) hosted by the Groningen Institute for Evolutionary Life Sciences (GELIFES) is located in the very heart of intensive crop and livestock production in Europe. This implies that the region is an important cornerstone for food security nationwide. In general, intensive agricultural production faces several problems, among others the contamination of animal products and manure with antibiotics, and the enrichment of groundwater with nitrate, which limits its use as drinking water. In both cases fertilizer strategies offer an efficient way to tackle these challenges efficiently. In the first case, antibiotics could be replaced at least partially by feed supplementation with phytopharmaceuticals, in particular glucosinolate-containing crop plants. High glucosinolate contents are hereby warranted by a sufficiently high sulfur supply. In the latter case, an efficient reduction of nitrogen losses will only be feasible if the nitrogen input by mineral and organic fertilizers is limited. Then advanced technological equipment in the field of precision agriculture and personal cultivation know-how is required for a site- and crop-specific nutrient management, which maintains a high production level without compromising food and environmental quality. Here again, a sufficiently high sulfur supply is essential to ensure nitrogen utilization efficiency on arable and grassland. But there is more to the major plant nutrient sulfur, for example its role in resistance against biotic and abiotic stress. At this point basic fertilizer research needs to go hand in hand with physiological and molecular studies in order to identify regulatory mechanisms so that it is possible to exploit the full potential of an adapted sulfur supply, which can exceed the physiological crop demand under stress conditions. International experts who look back to 28 years of sulfur research in the field of fundamental, environmental and agricultural aspects came to Groningen in order to present latest findings and to outline visionary future directions. At this point we would like to thank the local organizers who made the 25th CIEC Symposium an unforgettable event for all participants!

Ewald Schnug
President of CIEC

Silvia Haneklaus
Secretary General of CIEC

Modulation of content and composition of glucosinolates in *Brassica* upon abiotic stress

Tahereh A. Aghajanzadeh[1] and Luit J. De Kok[2]
[1]Department of Biology, Faculty of Basic Science, University of Mazandaran, Babolsar, Iran (E-mail:t.aghajanzade@umz.ac.ir): [2]Laboratory of Plant Physiology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, P.O. Box 11103, 9700 CC Groningen, the Netherlands

Plants elicit multiple responses when exposed to a variety of biotic and abiotic stress factors. These stress factors induce signaling cascades that activate ion channels, kinases, production of reactive oxygen species (ROS), and accumulation of plant hormones. These signals affect eventually both the primary and secondary metabolism resulting in a substantial variation in the plant metabolome. Plant secondary metabolism shows a high phenotypically plasticity in response to both biotic and abiotic stress factors. Glucosinolates are secondary sulfur compounds, which occur in high levels in Brassica vegetables and which are responsible for their characteristic flavor and odor and maybe involved in the defense against insects and pathogens. Moreover, they also have high nutraceutical and pharmacological value. Currently more than 130 different glucosinolates have been identified in plants and more than 30 of them are present in Brassica species. Their content and composition in plants is strongly affected during plant development and is affected by various environmental factors, *viz.* nutrient availability (S, N, K, Se and B) temperature, drought, UV-B, as well as fungal and bacterial pathogens. In the current study, the impact of chloride and sulfate salinity and high levels of Cu, Zn and Ni on the content and composition of glucosinolates was investigated in seedlings of *Brassica* species.

Sulfate is an important trigger of abscisic acid biosynthesis and stomata closure

Sundas Batool[1], Veli Vural Uslu[1], Hala Rajab[1], Cornelia Herschbach[2], Heinz Rennenberg[2], Dietmar Geiger[3], Rainer Hedrich[3], Rüdiger Hell[1] and Markus Wirtz[1]
[1]Centre for Organismal Studies (COS), Heidelberg University, Im Neuenheimer Feld 360, 69120 Heidelberg, Germany (E-mail: markus.wirtz@cos.uni-heidelberg.de); [2]Institut für Pflanzenbiologie, Technische Universität Braunschweig, Humboldtstraße 1, 38106 Braunschweig, Germany; [3]Julius-von-Sachs-Institut für Biowissenschaften, Molekulare Pflanzenphysiologie und Biophysik, 97082 Würzburg, Germany

Sulfur is an important macronutrient of all plants. Its uptake in form of sulfate from the soil is facilitated by plasma-lemma localized high-affinity sulfate transporters that are specifically regulated in response to internal demand and environmental challenges. Surprisingly, sulfate has been reported as the first metabolite whose concentration in the xylem is altered upon drought in the xylem. It was also shown to affect stomata closure by serving as substrate and activator of the quick anion channel 1 (Ernst et al. 2010; Malcheska et al. 2017). Furthermore, the external sulfate supply is known to impinge on ABA steady state level in Arabidopsis seedling (Cao et al. 2014).

In the current study, we uncover the molecular mechanism of sulfate-induced stomata closure and provide *bona fide* evidence for the role of sulfate in ABA biosynthesis: We show that feeding of physiologically relevant sulfate concentrations via the petiole trigger stomata closure by induction of plasma-lemma localized NADPH oxidases that produce reactive oxygen species (ROS). Since ROS production is a known response of stomata towards ABA stimulus, we tested if sulfate can promote stomata closure and ROS production in the ABA biosynthesis mutant, *aba3*, and the ABA insensitive mutant, *abi2*. Sulfate failed to induce stomata closure and production of ROS in both mutants. Furthermore, loss of the ABA-activated slow anion channel 1 (SLAC1) impaired sulfate-induced stomatal closure. Application of the ABAleon2;1 sensor, a FRET-based probe for non-invasive determination of ABA by live cell imaging, ultimately proved that sulfate application rapidly increases cytosolic ABA levels in guard cells. In view of the fact that the molybdenum cofactor sulfurylase ABA3 uses cysteine as substrate for activation of ABA biosynthesis (Bittner et al. 2001), we tested the ability of sulfate to close stomata of the *sir1-1* and the *serat tko* mutants, which are both impaired in cysteine biosynthesis. As expected, sulfate was unable to induce stomata closure and ROS production in these mutants. In agreement with a promoting role of cysteine in ABA synthesis, application of ABA or cysteine to *sir1-1* and *serat tko* induced ROS production and stomata closure. The latter results proved that ABA signaling is still functional in both cysteine synthesis depleted mutants. Intriguingly, guard-cell autonomous production of ABA by ABA3 was sufficient for sulfate-induced stomata closure. Taken together our results promote sulfate as a xylem-delivered root-to-shoot signal that upon early drought induces ABA production in guard cells resulting in stomata closure.

Bittner, F., Oreb, M., and Mendel, R.R. (2001) ABA3 Is a molybdenum cofactor sulfurase required for activation of aldehyde oxidase and xanthine dehydrogenase in *Arabidopsis thaliana*. J. Biol. Chem. 276: 40381-40384.

Cao, M.J., Wang, Z., Zhao, Q., Mao, J.L., Speiser, A., Wirtz, M., Hell, R., Zhu, J.K., and Xiang, C.B. (2014) Sulfate availability affects ABA levels and germination response to ABA and salt stress in *Arabidopsis thaliana*. Plant J. 77: 604-615.

Ernst, L., Goodger, J.Q.D., Alvarez, S., Marsh, E.L., Berla, B., Lockhart, E., Jung, J., Li, P., Bohnert, H.J., and Schachtman, D.P. (2010) Sulphate as a xylem-borne chemical signal precedes the expression of ABA biosynthetic genes in maize roots. J. Exp. Bot. 61: 3395-3405.

Malcheska, F., Ahmad, A., Batool, S., Müller, H.M., Ludwig-Müller, J., Kreuzwieser, J., Randewig, D., Hänsch, R., Mendel, R.R., Hell, R., Wirtz, M., Geiger, D., Ache, P., Hedrich, R., Herschbach, C., and Rennenberg, H. (2017) Drought enhanced xylem sap sulfate closes stomata by affecting ALMT12 and guard cell ABA synthesis. Plant Physiol. in press.

Contamination of organic waste materials by antibiotics and its implications for use in agriculture

Elke Bloem, Lennart Lehmann, Ulrike Timmerer, Silvia Haneklaus and Ewald Schnug
Institute for Crop and Soil Science, Julius Kühn-Institut (JKI), Braunschweig (E-mail: elke.bloem@julius-kuehn.de)

Organic wastes are used increasingly in biogas plants to produce energy and digestates are used as fertilizers. Input and output materials were collected from 30 biogas plants comprising manures originating from different animal species, and sewage sludge in varying ratios. Eight representative antibiotics were determined: sulfadiazine (SFD) and sulfamethazine (SMZ), enrofloxacin (EN), ciprofloxacin (CF), difloxacin (DF), chlortetracycline (CTC), oxytetracycline (OTC) and tetracycline (TC). Input and output materials of biogas plants were sampled at the same day. All sewage sludge samples proved to be contaminated by antibiotics. In cattle manure OTC, TC and EF were prevailing in higher concentrations, in pig slurry SFD was found often, too. In poultry dung the dominating antibiotics were EF, CF and TC. The highest antibiotic concentrations with values > 8600 µg EF/kg DM were detected in chicken manure. The highest concentration of 8626 µg EF/kg DM and 8180 µg TC/kg DM was found in in poultry dung, followed by 7781 µg OTC/kg DM in pig slurry.

The composition of antibiotics in sewage sludge differed from that in animal manures: CF and TC were detected in all sewage sludge samples. EF and SFD were detected frequently. In all sewage sludge samples TC was found while in manures and slurries OTC was the dominating tetracycline. Comparable to the input materials digestates contained significant amounts of antibiotics. All digestates derived from sewages sludge were contaminated with a maximum value of 2440 µg TC/kg DM. 79% of all substrates (animal manures + sewage sludge) and 86 % of the digestates contained antibiotics. The data showed that antibiotics were hardly degraded during the fermentation process in the biogas plant. The results revealed that the direct use of farmyard manure and sewage sludge will yield a contamination with antibiotics comparable to that of digestates.

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The incorporation of elemental sulfur as fertilizer coating material increased yields of wheat crop

D.L. Bouranis[1*], D. Argyros[3], G. Georgoulas[3], D. Petrakos[3], A. Varnas[3], D. Gasparatos[2], M. Margetis[1], F. Maniou[1], S.N. Chorianopoulou[1], H. Mavrogiannis[3] and D. Benardos[3]
[1]Plant Physiology and Morphology Laboratory, Crop Science Department, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece (E-mail: bouranis@aua.gr); [2]Soil Science Laboratory, Department of Hydraulics, Soil Science and Agricultural Engineering, School of Agriculture, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; [3]Sulphur Hellas S.A., Leoforos Athinon 142, 104 42 Athens, Greece

In a preliminary set of field trials, a number of 11 crops of durum wheat (Simeto, Meridiano and Quantrato cultivars) were established in different areas of central and northern Greece and soil fertility levels was determined prior to crop establishment. Sowing and harvesting took place mid November 2015 and end of June 2016, respectively. Each crop (roughly 2-3 acres each) was divided into two parts: one of them served as the reference crop that received conventional fertilization scheme, whilst the other one (the treatment SG-crop) received the same combination of fertilizers additionally coated with 2% elemental sulfur (Sulfogrow® by Sulphur Hellas S.A.).

Six of the fields were containing no calcium carbonate or traces of it, with pH ranging from 6.20 to 7.74. In five of them, all characterized by adequate fertility levels, the yields of the SG-crops presented relative increases from 8% to 27%. In the field characterized by very low soil initial phosphate content coupled with low potassium content, the yield of the SG-crop presented a relative decrease by 11%.

The other five fields were containing moderate or high calcium carbonate, with pH ranging from 7.96 to 8.20. In three of them, those with adequate fertility levels, the yields of the SG-crops presented relative increases from 3% to 8%. The other two cases of this category presented relative decreases in the yield by 27% and 3.5%, respectively. The rhizospheric soil of these cases was characterized by very low initial phosphate content coupled with marginal iron content, whilst in the former one the rhizospheric soil additionally presented a very low concentration of humic substances and very low sand content.

PGRP response to elemental sulfur coated fertilizers application in a calcareous soil under wheat cultivation

D.L. Bouranis[1], A. Venieraki[2], M. Margetis[1], S.N. Chorianopoulou[1], F. Maniou[1], B. Zechmann[3], D. Gasparatos[4], H. Mavrogiannis[5], D. Benardos[5] and P. Katinakis[2]
[1]Plant Physiology and Morphology Laboratory, Crop Science Department, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece (E-mail: bouranis@aua.gr); [2]General and Agricultural Microbiology Laboratory, Crop Science Department, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece; [3]Center for Microscopy and Imaging, Baylor University, One Bear Place 97046, Waco, TX 76798-7046, USA; [4]Soil Science Laboratory, Department of Hydraulics, Soil Science and Agricultural Engineering, School of Agriculture, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; [5]Sulphur Hellas S.A., Leoforos Athinon 142, 104 42 Athens, Greece

A durum wheat crop established in Central Greece, served as the reference crop that received conventional fertilization scheme, whilst the nearby field (0.9 acres each) received the same combination of fertilizers additionally coated with 2% elemental sulfur (the treatment SG-crop; SG stands for Sulfogrow® produced by Sulphur Hellas S.A.). Soil testing revealed that the SG-crop was established in a field with low soil fertility compared to the reference field. Each field was divided into five parts and nutrient dynamics in crop's aerial part was monitored during cultivation period, along with fertility dynamics and microbial population dynamics in crop's rhizosphere.

Dry mass accumulation and plant robustness were the same despite the significant difference in rhizosphere's characteristics, whilst yield of SG-crop was 3.5% less. Iron concentration in the aerial part was significantly higher especially 20 days after the additional fertilization at days 100 and 110 after showing. Total colonies found to be significantly higher from day 60 onwards and among them the colonies that presented sulfatase activity were prominent, especially after the additional fertilization. In the rhizosphere of reference crop the following species have been identified: *Pseudomonas orientalis, P. libanensis, P. fluorescens, P. moraviensis* and *P. putida*, whilst in the rhizosphere of the SG-crop the identified species included *Pseudomonas fluorescens, P. azotoformans, P. reactans, P. libanensis, P. koreensis, Xanthomonas sp., Bacillus sp.* and *Paenibacillus polymyxa*, along with *Cellulosimicrobium cellulans,* and *Cellulomonas sp.*

Our data support the hypothesis that the elemental sulfur coating of the applied fertilizers significantly boosted the action of the above mentioned plant growth promoting rhizobacteria, which in turn contributed to the mobilization of immobilized sulfate, phosphorus, iron and manganese in quantities capable to support the SG-crop.

The effect of expression levels of *AtGR1* and *AtGR2* genes on the sulfur assimilation pathway in *Arabidopsis thaliana* plants

Aner Cohen and Rachel Amir
Migal Research Institute, Tel Hai Collage, Tarshish 1, 11016 Kiryat Shmona, Israel (E-mail: rachel@migal.org.il)

Sulfur is an important element in plant nutrition required for the synthesis of several essential metabolites such as amino acids, *viz.* cysteine and methionine, and glutathione. Glutathione (GSH) plays diverse roles in plants, including preventing oxidation damage. During oxidative stress, two oxidized GSH molecules form a glutathione disulfide complex through a disulfide bond (GSSG). These complexes are degraded in vacuole or are recycled by reduction, which is catalyzed by the enzyme glutathione reductase (GR) in a process that requires NADPH as an electron donor. In plants, two genes encode for GR: GR1, which is located in the cytosol; and GR2, which is located in the chloroplast. It was expected that the overexpression of GR would lead to a higher GSH content and tolerance to oxidative stress. However, the effect was deceptive, since several plants were indeed more tolerant, while others were not, and some were more sensitive.

Transgenic *Nicotiana tabacum* plants over-expressing GR1 and GR2 genes from *Arabidopsis thaliana* (*AtGR1* and *AtGR2*) showed high GR activity and elevated cysteine and GSH levels, together with a high GSH/GSSG ratio, but they did not differ from wild type plants regarding their tolerance to oxidative stress. The high GSH levels in these plants might result from higher GR activity that reduced its degradation, but it cannot explain the higher cysteine levels. Thus, we assume that the elevation in these two metabolites results from GR overexpression that increased cysteine synthesis through the sulfur assimilation pathway. A high level of cysteine enables the accumulation of GSH, as previously shown. The hypothesis of this research proposed (as theoretically previously suggested) that when GSH donates two electrons to the catalytic reaction of adenosine 5' phosphosulfate reductase (APR), which is known to be the rate-limiting enzyme sulfur assimilation pathway, GSH is oxidized, and thus GR is required to reduce GSSG to GSH in order to maintain APR activity and make this pathway efficient.

To examine this possibility further, these two genes were overexpressed in Arabidopsis plants, which, together with *gr1* and *gr2* mutants, were compared to wild type plants and transgenic plants having an empty vector. The results showed that compared to the control plants, plants overexpressing GR have a higher level of GR activity, a high GSH/GSSG ratio, and higher contents of cysteine, glutathione and methionine, while the mutants exhibit lower levels in all of these parameters. The levels of sulfide, the product of APR, increased significantly in transgenic plants but decreased significantly in the mutants; however, the levels of sulfate did not change compared to the control in transgenic plants but increased in the mutants. These findings support the hypothesis that GR plays an important role in the sulfur assimilation pathway in Arabidopsis and apparently in other plants. In addition, this work offers a new way of enhancing a plant's nutritional value by increasing the levels of both sulfur amino acids, keeping the level of glutathione high for its different functions.

Phosphorus budgets and bioavailable phosphorus content in soil - results of a long-term field experiment

Bettina Eichler-Löbermann, Theresa Zicker, Katrin Wacker and Ralf Uptmoor
University of Rostock, Chair of Agronomy and Crop Science, J. von Liebig Weg 6, 19059 Rostock, Germany (E-mail: bettina.eichler@uni-rostock.de)

Due to the scarcity of mineable phosphorus (P) an efficient use of P is a main objective in sustainable agriculture. An application of P adapted to crop requirements and the nutrient recycling with wastes and residues can help to achieve this aim. The content of bioavailable P in soil can be used to evaluate the current soil P status and to calculate the fertilizer need. However, the availability of P in soil depends not only on the P input but is also regulated by biological and biochemical processes in soil and environmental conditions. Long-term field experiments can give an extensive overview about the effectiveness of P application also considering the influence of annual environmental conditions, P mobilization and translocation processes in soil. At the Rostock long-term field experiment the effect of single and combined P treatments are investigated since 1998.

The long-term field experiment is located in the northeast of Germany and influenced by a maritime climate (600 mm annual rainfall; 8.1°C annual temperature). The initial double-lactate soluble P content (Pdl) of the soil was 42.2 mg kg^{-1} indicating a sub-optimal P supply according to the German soil P test classification. The trial was designed as a randomized split plot experiment in four replicates. The main plots comprised three organic P treatments (no P, biowaste compost, and cattle manure). The subplots comprised three inorganic P treatments (no P, Triple-super-P (TSP), and biomass ash). Compost and manure were applied at rates of about 30 t ha^{-1} in September every three years beginning in 1998. TSP and biomass ash were applied annually at rates of 21.8 to 30.0 kg P ha^{-1}. Soil and plant sampling was done twice a year and a broad spectrum of soil characteristics, crop yields and crop P uptakes were investigated.

The different P treatments and their combinations resulted in different crop P uptakes and P budgets (Table 1). These management effects also influenced the bioavailable P contents in soil (measured as Pdl; Table 2). Highest contents of Pdl were measured in the combined fertilizer treatments with compost and inorganic fertilizers (about 55 mg kg^{-1} soil). Compost application resulted in higher Pdl contents than manure application, which could be explained by the relatively high percentage of mineralized P in compost and its stable organic compounds. The organic fertilizers had a comparable P fertilizer effect as the inorganic fertilizers. Close significant ($p<0.05$) correlations between the P budgets and the Pdl concentrations in soil in the particular periods were calculated ranging from 0.73 to 0.98. However, the data also showed fluctuations of the Pdl values during the experimental period, which cannot be explained only by the P supply. Here, we expect a decisive role of biological and chemical P turnover processes which may be affected by weather conditions. It can be concluded that P budgets can be used to predict changes in soil test P over long periods under field conditions but may be unsuitable to reflect the current soil P status.

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Table 1. Total P supply, total crop P uptake (P removal) and total P budget accumulated after 17 years in field experiment Rostock

Treatment	Total P supply	P removal (kg ha^{-1})	P budget
Control	0	422	-422
TSP	379	467	-88
Ash	348	439	-91
Manure	391	465	-74
Man + TSP	770	467	303
Man + Ash	739	482	257
Compost	396	470	-74
Com + TSP	775	498	277
Com + Ash	744	508	236

TSP = Triple-Superphosphate, Manure/Man = Cattle manure,
Compost/Com = Biowaste compost, Ash = Biomass ash

Table 2. Contents of bioavailable P (Pdl, mg kg^{-1} soil) as average of three-year periods and over the whole experimental time from 1998 to 2015 in the Rostock field experiment

Treatment	1998-2000	2001-2003	2004-2006	2007-2009	2010-2012	2013-2015	Mean
Control	35.7a	41.1a	36.6a	32.3a	30.4a	28.8a	34.a
TSP	41.7bc	51.4cd	48.1c	40.1b	38.8b	36.7b	43.8c
Ash	36.1a	46.7b	44.6b	41.4b	37.0b	36.8b	41.b
Manure	40.7b	50.0c	48.9cd	40.5b	37.1b	37.4b	43.5c
Man + TSP	42.2bc	58.7e	54.2f	47.2c	44.6d	44.6d	49.e
Man + Ash	46.8de	51.8cd	51.8ef	44.8c	41.7c	43.3cd	47.d
Compost	44.3cd	53.1d	51.1de	46.8c	45.2d	41.2c	47.d
Com + TSP	48.0e	58.8e	61.1g	54.9d	53.2e	49.5e	55.3f
Com + Ash	51.2f	59.3e	60.3g	54.6d	51.3e	49.2e	55.2f
Mean	43.0	52.3	50.8	44.7	42.1	40.8	

Different letters indicate significant different means between fertilizer treatments, Duncan-test P ≤ 0.05; TSP = Triple-Superphosphate, Manure/Man = Cattle manure, Compost/Com = Biowaste compost, Ash = Biomass ash, each period consists of three single years, soil sampling was done every single year

Effects of sulfur and manure levels on yield and yield components of canola (*Brassica napus* L. *var*. Okapi) on a calcareous soil in Iran

Manoochehr Farboodi, Fariborz Jalali, Naser Nazari, Hosein Besharati and Sharam Shahrokhi
Soil Science Department, Agricultural College, Miyaneh Branch, Islamic Azad University, Tehran, Iran (E-mail: farboodi1962@gmail.com)

This research was conducted to study the effect of different levels of sulfur and manure on yield and yield components of canola (*Brassica napus* L. *var.* Okapi) in a two-year experiment (2015-2016). The field experiment had a completely randomized block design consisting of a 3 × 3 factorial combination of three sulfur rates (0, 152.5 and 305 kg S ha^{-1}) and three manure rates (0, 10, 20 ton ha^{-1}) in threefold replication. The following plant characteristics were determined: plant height, number of seeds per pod, weight of pods, weight of 1000 seeds, number of pods, number of lateral branches, economic yield, biomass, oil and glucosinolate content. The results of the ANOVA revealed that the sulfur rates had a significant influence on plant height, number of seeds per pod, weight of 1000 seeds ($P <0.01$). The manure levels influenced the number of seeds per pod ($P <0.01$) and the weight of thousand seeds ($P <0.05$) significantly. Sulfur × manure interactions were significant with respect to the oil content, number of pods ($P < 0.05$), glucosinolate content, lateral branches and biomass ($P <0.01$). The data showed that 20 ton ha^{-1} manure together with 305 kg ha^{-1} sulfur resulted in the highest yield and oil content of canola with the lowest glucosinolate content in the Miyaneh region of Iran.

Appraisal of fertilizer practices alleviating stress conditions

Silvia Haneklaus, Elke Bloem and Ewald Schnug
Institute for Crop and Soil Science, Julius Kühn-Institut, Federal Research Institute for Cultivated Plants, Braunschweig, Germany (E-mail: silvia.haneklaus@julius-kuehn.de)

Plants encounter varies forms of biotic and abiotic stress from sowing to harvest. Accordingly, fertilizer applications have been developed to strengthen the resistance of plants against various stressors. Instinctive management practices feeding plants can be traced back to the Neolithic agricultural revolution. In the 19th century plant nutrition became an area of research in the field of agricultural chemistry. Liebig's 'Law of the Minimum' (1855) still is the basic principle of plant nutrition. It states that the exploitation of the genetically fixed yield potential of crops is limited by the variable, which is insufficiently supplied to the greatest extent. With view to abiotic and biotic stress factors this postulation should be extended by the phrase 'and/or is impaired by the strongest stress factor'. Interactions between mineral elements and plant diseases are well known for essential macro and micro plant nutrients, and aluminum and silicone. The concept of Sulfur Induced Resistance (SIR) is meanwhile acknowledged in plant pathology and targeted sulfur fertilization is a backbone for promoting growth and plant health in intensive cropping systems. In comparison, the potential of fertilization to alleviate abiotic stress has not been compiled in a user-orientated manner. Abiotic stress factors comprise nutrient and water deficiency, soil pH, temperature, oxygen supply, mechanical pressure, injury and chemical compounds. Though various essential macro- and micro-nutrients are involved in tolerance mechanisms against abiotic stress, only a limited number of elements proved to alleviate stress conditions under field conditions. It is the objective of this contribution to summarize the influence of nutrient deficiency in general and the nutritional status of sodium, potassium and silicon in particular on resistance of crop plants against abiotic stress factors such as drought, salinity and frost. In addition, the significance of seed priming with various nutrients for tolerance against abiotic stress is discussed.

Linking genes to field performance: adventures in sulfur research

Malcolm J. Hawkesford
Rothamsted Research, West Common, Harpenden, Hertfordshire, AL5 2JQ, U.K. (E-mail: malcolm.hawkesford@rothamsted.ac.uk)

The development of widespread sulfur deficiency in crops became evident in the 1980's and 90's. It is well documented that this arose due to reduced aerial inputs due to decreased pollution as well as the use of low S fertilizer formulations. Negative impacts were seen on high S-requiring crops such as *Brassica napus* (oilseed rape, colza, canola) and also for cereals such as wheat. Both yield and quality were affected, and for example a detrimental impact of S deficiency on the content of S-rich storage proteins of wheat, which are essential for dough rheology, was observed (Zhao et al. 1999). In addition, low S availability has substantial impacts on uptake and accumulation of Se and Mo, which may have substantial implications for nutritional quality (Shinmachi et al. 2010; Stroud et al. 2010). Many studies demonstrated that remedial application of S fertilizers successfully restored yield and quality parameters. One area of importance was accurate diagnosis (Blake-Kalff et al. 2000; Blake-Kalff et al. 2001). Remediation of crop S deficiencies was a recent success story in relation to applied plant nutrition studies coupled with appropriate agronomy.

More fundamental studies have focused on the sulfur uptake and assimilatory pathways and their regulation as a paradigm for plant and indeed crop responses to mineral nutrition (Hawkesford and De Kok 2006). The rationale for such studies is a genetic solution for producing crops with increased efficiency for uptake and utilization of S, or for reduced physiological requirements for S. The most significant advances at the molecular level have been achieved using the model plant Arabidopsis thaliana, although such studies have more recently been extended into a number of crops including rice, wheat and Brassica, aided by rapid recent advances in resources for genomics in these crops (Buchner et al. 2004). Achievements over the past 20 years have resulted in the elucidation of molecular aspects of uptake and assimilation pathways, the existence of large multigene families, with isoforms having defined roles, pathways for regulation and inter connections with a range of metabolic pathways and physiological responses. A suggestion that manipulation of feedback mechanisms which restrict uptake could enable luxury uptake during time of plenty (immediately following fertilizer applications) and subsequent effective internal management of this reserve S, could ensure the most effective use of fertilizer applied S. A wide range of roles and requirements for adequate S-nutritional status have been established indicating little opportunity for decreasing crop S demands. One exception has been in identifying prospects for improving effective remobilization of S within the plant particularly, between senescing and newly developing organs (Blake-Kalff et al. 1998; Dubousset et al. 2009). Brassica species have proved to be a useful model for understanding physiological aspects of regulation, having a high S requirement and being particularly amenable to experimental manipulation of S sources for growth (Aghajanzadeh et al. 2016; Hawkesford and De Kok 2006).

Recent developments in high resolution crop monitoring and phenotyping offer opportunities to closely monitor impacts of S nutrition on crop growth and development, and hence performance in the field. As well as being able to follow crop establishment and determine yield components, opportunities exist to monitor subtle effect of fertilizer on developmental processes such as flowering or other physiological factors such as resistance to stress. Application of an automated field phenotyping platform to monitor crop performance at Rothamsted Research will be presented.

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Distinct microbial processes and functions of maize stalk- and fertilizer-N in arable soil

Hongbo He[1,2], Guoqing Hu[1,3] and Xudong Zhang[1]
[1]Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China (E-mail: hehongbo@iae.ac.cn); [2]National Field Observation and Research Station of Shenyang Agroecosystems, Shenyang 110016, China; [3]National Engineering Laboratory for Efficient Utilization of Soil and Fertilizer Resources, College of Resources and Environment, Shandong Agricultural University, Taian 271018, China

Crop residue returning is one of the key practices for the improvement of soil fertility and consequently the sustainability of agroecosystems. Under this management, both fertilizer and crop residue derived nitrogen (N) are important anthropogenic N sources for microbial immobilization in arable soils. However, how N applied with these different sources is dynamically involved in microbial-driven N cycling in the soil-crop system remains unclear. A field experiment with annual maize stalk mulching was conducted in an Alfisol of a temperate agro-ecosystem but only the first-year applied chemical fertilizer and maize stalk were crossly ^{15}N-labeled. Compound-specific ^{15}N enrichment in soil amino sugars was temporally measured to gain insights into microbial immobilization of fertilizer and maize stalk derived N and furthermore the involvement of extraneous N in soil N retention. The initial transformation (in the first year) of fertilizer-N into amino sugars was much more rapid than maize stalk-N, but the eventual accumulation of maize stalk-N in amino sugars was significantly larger than fertilizer-N over five experimental years. Throughout the experiment, most of the residue N was retained in soil, compared to the less proportion of fertilizer N in soil (73.8% vs. 40.9%). Simultaneously, the contribution of microbial residues to maize stalk-N retention in soil was significantly larger than those of fertilizer-N, implying the higher stability of maize stalk-N in soil matrix. Therefore, the anthropogenic N in different forms played distinct functions in N cycling in soil-crop system. Maize stalk-N was expected to play an important role in building up and sustaining long-term N reserve in the arable soil, being as an important foundation for effective crop uptake of reactive fertilizer-N.

OAS responsive repressor proteins linking sulfate metabolism and glucosinolate biosynthesis

Rainer Hoefgen
Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, D-14476 Potsdam-Golm, Germany (E-mail: hoefgen@mpimp-golm.mpg.de)

The *SDI1* and *SDI2* genes have been identified in early transcriptomics studies as being highly expressed in response to sulfate depletion in Arabidopsis and wheat. Later we linked their induction to the accumulation of *O*-acetyl-serine (OAS), which highly accumulates in response to reduced sulfate availability, but also in response to other stresses. Both genes belong to a cluster of OAS responsive genes. We identified that in Arabidopsis SDI1 (At5g48850) and SDI2 (At1g04770) are involved in down-regulating glucosinolate biosynthesis. Overexpression of both, *SDI1* and *SDI2*, result in a reduced accumulation of aliphatic and to a lesser extent indolic glucosinolates. We could show that this is achieved through a direct protein-protein interaction of SDI1 with the transcription factor MYB28. This complex prevents the transcription of genes controlled by MYB28, previously identified to play a role in controlling glucosinolate biosynthesis. SDI1 and SDI2, thus, down-regulate the expression of the glucosinolate pathway controlling transcription factors MYB29 and MYB76, and MYB28 itself and, hence, their downstream target genes. As glucosinolates provide a substantial sink for sulfate this regulatory step allows plants under sulfate starvation conditions to reduce or stop *de novo* glucosinolate biosynthesis in favor of plant primary metabolism.

Assessing phosphorus extractability by Olsen P and AL-P tests in acid soils after P fertilization with compost versus mineral fertilizer

Carmo Horta[1,2]
[1]Instituto Politécnico de Castelo Branco, Escola Superior Agrária, Quinta da Sra. de Mércules, 6001- 909, Castelo Branco, Portugal; [2]CERNAS, Research Centre for Natural Resources, Environment and Society, Quinta da Sra. de Mércules, 6001-909 Castelo Branco, Portugal (E-mail: carmoh@ipcb.pt)

Soil P tests, like the Olsen (Olsen-P, Olsen et al., 1954) and Ammonium Lactate (AL-P, Egnér et al., 1960) methods, are useful tools to assess P phytoavailability. Such methods should provide accurate agronomic meaning in order to achieve a sustainable P fertilization. The properties of composts, namely the organic matter content together with the P-chemical forms in the fertilizers, could influence soil P sorption after fertilization and consequently change the amount of P extracted by such soil P tests. Using the same rate of P fertilization applied by different fertilizers, e.g. compost (CP) or single superphosphate (SSP) we hypothesized that Olsen and AL-P methods are able to discriminate differences in the soil P availability induced by fertilizer properties. So, the main objective of this work was to evaluate the accuracy of two soil tests commonly used in Europe, the Olsen and AL-P methods, in assessing P availability after application of compost or single superphosphate to a low-P acid soil. To do so, an incubation experiment was performed over 140 days. The soil used was a low-P dystric Regosol and the fertilizer used was a compost obtained through aerobic composting of sewage sludge mixed with sawdust (CP) and the single superphosphate (SSP). The experimental design was completely randomized with two fertilizers, four P application rates and four replicates of each treatment generating a total of 32 incubation boxes. The P application rates (kg P ha^{-1}) were: 6.5, 13, 26 and 52. The amount of compost used was constrained by the levels of N sustainable fertilization with a maximum application rate of 170 kg N ha^{-1} from organic amendments. Therefore, P application rates of 26 and 52 kg ha^{-1} were achieved through the addition to the compost of SSP: the rate of 26 kg P $ha^{-1}$was set by adding 19.5 kg P ha^{-1} in the form of SSP to the compost, and the 52 kg P ha^{-1} rate by adding 39 kg P ha^{-1} in the form of SSP. The compost and the SSP were finely ground to 0.5 mm and sieved before applying to the soil, and maintained in the dark at 25 ^{o}C and at 70% field capacity. After incubation, a composite soil sample was taken of each treatment and replicates, and analyzed for AL-P and Olsen-P. P forms in the compost and in the SSP were also analyzed by a sequential fraction method (Traoré et al. 1999): the first extraction was performed with H_2O (H_2O-P), the second with 0.5 M $NaHCO_3$ (pH 8.5; $NaHCO_3$-P), the third with 0.1 M NaOH (NaOH-P) and the fourth with 1 M HCl (HCl-P). P in the inorganic forms H_2O-Pi or $NaHCO_3$-Pi is considered to be easily available to crops; the NaOH-Pi is considered to be mainly bound to Fe and Al oxides or metal-organic complexes, and thus evaluated as being moderately labile and the HCl-Pi is regarded as being bound mainly to Ca in low-solubility precipitates, such as apatite or octacalcium phosphate, and thus represent stable P forms (Traoré et al., 1999; Gagnon et al., 2012). The total dissolved P in the extracts of the first three fractions was also analyzed by acid-potassium persulfate digestion (American Public Health Association, 2012) and the dissolved organic P (Po) was calculated as the difference between the total dissolved P and inorganic P quantified in each fraction. In all the cases, orthophosphate P in solution was determined by the molybdate blue method of Murphy and Riley (1962). The compost used in this work has an organic matter content of 640 g kg^{-1} (dry matter) with a C/N = 27. CP has 0.42 g P kg^{-1} with 80% of this total amount of P in inorganic forms (Pi). SSP has 94 g kg^{-1} of Pi. CP had almost 50% of the total amount of Pi in forms easily available to crops and 37% in forms considered moderate labile, mainly bound to Fe and Al oxides or metal-organic complexes. Contrary to this, in SSP the forms of Pi considered easily available to crops represent almost 86% of the total amount of P, with a trace amount of NaOH-Pi fraction. In addition, The HCl-Pi fraction was similar between CP (15%) and SSP (13%). After 140 days of soil incubation with the fertilizers the amount of P extracted by AL-P and Olsen methods showed to be significantly higher after CP treatments in relation to SSP at the same P rate. Even applying simultaneously compost and SSP, there was a significant increase in soil P availability relatively to the SSP treatments. From these observations, we conclude that the organic matter content of CP might have prevented soil P sorption, which further explains the higher P availability in all CP treatments. Thus, both methods were able to discriminate differences of soil P availability caused by fertilizer properties.

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Acidification of digestate with sulfuric acid: interests for researchers and farmers

Brian H. Jacobsen[1], Fabrice Marcovecchio[2], Ivona Sigurnjak[3], Caroline Leroux[2], Francis Astier[2] and Christophe Fourcans[2]
[1]IFRO, University of Copenhagen, Rolighedsvej 25, DK-1958 Frederiksberg C, Denmark; [2]Le Laboratoire Départemental d'Analyses et de Recherche (LDAR), 180 rue Pierre-Gilles de Gennes, Barenton-Bugny, 02007 Laon cedex, France (E-mail: fmarcovecchio@aisne.fr); [3]Ghent University, St. Pietersnieuwstraat 33, B-9000 Ghent, Belgium

Acidification is widely used only in Denmark for several years and the regulatory incentives have helped to implement this technology. VERA test in Denmark is in reality not used by other countries in their assessment of the technology. For digestate acidification or acid use, the following Danish, French and German regulations are established: Agriculture and Market control, Environment, French Standards, European regulation for animal by products and European regulation for diseases control (bovine tuberculosis and paratuberculosis, influenza). There is an interest in digestates acidification by using H_2SO_4 to reduce NH_3 losses and explore: i) impact on microbiological parameters at pH 5.0 with or without additives in digestates; ii) impact on sulfur, pH and others physico-chemical parameters in the soils. This works are an issue from the INEMAD project. This project has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 289712 (www.inemad.eu). The INEMAD project has a distinct focus on management strategies to improve the use of nutrients from manure and digestate in European agriculture. This research aims at reviewing the current acidification in selected areas in order to propose its improvement in the exchange and local use of digestate as an organic fertilizer. High livestock density is found in the Flemish region of Belgium, the Netherlands and the parts of France and Italy. Each year 2-5 million tons of organic fertilizers or raw materials are exported from Belgium and the Netherlands to France or Germany, and a further increase is expected.

Comparison of some element ratios of various Hungarian soil types

János Kátai and Imre Vágó
University of Debrecen, Faculty of Agriculture and Food Science and Environmental Management, Institute of Agricultural Chemistry and Soil Science, Böszörményi str. 138, H-4032 Debrecen, Hungary (E-mail: katai@agr.unideb.hu)

The residues of organic matter in the soil are transformed biologically over a shorter or longer period. In the humification process, a part of the organic decomposition products formed the dark-colored humus. The other part of the organic matter is also decomposed by the activities of heterotrophic organisms, in which inorganic substances are produced from the organic matter. The direction and intensity of the transformation, however, depends not only on the organic material stock of the soil, but also on the organisms in the soil, which are closely related to soil properties, natural fertility, environmental factors and applied agrotechnical processes. The rate and speed of humus formation and mineralization determine the soil organic matter stock and the set of nutrients that can be taken up by plants during the degradation of organic matter (Füleky and Rajkainé 1999).

Nutrient elements extracted from the soils with cultivated plants can be replaced by inorganic and/or organic fertilizers or other alternative nutrients that contribute to the soil fertility (Loch 1999). Soil organic matter has a primary important role in soil properties and preservation of fertility. Since significantly fewer organic and manure fertilizers are formed in the last few decades, there are countless experiments to replace the organic matter of soils. The transformation of alternative plant nutrient organic matter (composts, green manure, sewage sludge, slurry etc.) is determined by the living organisms with different activities in the soil.

In our publication the most important physical and chemical properties of 12 different soil types in eastern part of Hungary (chernozem, meadow, marsh, brown forest soil, blown sand, and solonetz) were analyzed. Vegetation of soil types were winter wheat, orchard, oak forest and natural grassland. There were investigated the available nutrient content of the soils (nitrate, $CaCl_2$ extractable phosphorus and sulfate content). There were also determined the total content (stock) of some elements (C, N, S, P) in the soils and were compared the ratios of the soils element ratios (C/N, C/S, N/S). Based on the results, there was also calculated the proportion of the available and the total element content of soils.

Growth parameters, nitrogen and sulfur uptake of onion as affected by different nitrogen fertilizers and nitrification inhibitor

Andrea Balla Kovács, Áron Béni, Rita Kremper and Evelin Juhász
Institute of Agricultural Chemistry and Soil Science, Faculty of the Agricultural and Food Sciences and Environmental Management, University of Debrecen, H-4032 Debrecen, Böszörményi Street 138. Hungary (E-mail: kovacsa@agr.unideb.hu)

A pot experiment was carried out to evaluate of the effect of different nitrogen fertilizers and the addition of a nitrification inhibitor on yield performance, nitrogen and sulfur uptake of onion (*Allium cepa)*. The study was conducted on humic sandy soil and consisted of 13 treatments in a randomized complete block design with four replications. Three types of nitrogen fertilizers (urea, ammonium nitrate, ammonium acetate) with two doses (120 kg N/ha, 240 kg N/ha) were applied. Nitrogen fertilizers were incorporated with or without nitrification inhibitor, Nitrapyrin (2-chloro-6-trichloromethyl-pyridine). The treatments were: 1. control; 2. urea (120 kg N/ha), 3. urea (240kg N/ha), 4. ammonium acetate (120 kg N/ha), 5. ammonium acetate (240 kg N/ha), 6. ammonium nitrate (120 kg N/ha), 7. ammonium nitrate (240 kg N/ha). In treatments 8. to13. all nitrogen fertilizers were supplemented with Nitrapyrin. All pots were supplied with standard dose of sulfur (40 kg S/ha) as K_2SO_4. Plant growth was monitored for three months. Leaf and bulb weights, size of bulbs and total plant biomass were measured. Nitrogen and sulfur content and uptake of onion also were determined. All of applied nitrogen fertilizers tended to increase the weight of leaves, but significant increasing effects were only measured by higher doses of urea, ammonium acetate and ammonium nitrate (240 kg N/ha) with a combination of Nitrapyrin compared to the control. The weight of bulbs significantly increased in treatments with higher dose of urea and ammonium nitrate (240 kg N/ha) compared to the control. The sizes of bulbs did not change in any treatment. The total plant biomass was the highest in the treatment of urea (240 kg N/ha) supplemented with Nitrapyrin. The urea, ammonium-acetate and ammonium nitrate enhanced the nitrogen content either in leaves, or in bulbs. The highest leaf nitrogen content was measured in the combined treatment of urea (240 kg N/ha) and Nitrapyrin, and the highest bulb nitrogen content was observed in the treatment of urea (240 kg N/ha) without Nitrapyrin. The total nitrogen uptake of onions was highest at a higher dose of urea (240kgN/ha), either with or without Nitrapyrin application. The nitrification inhibitor enhanced the total nitrogen uptake of plants when Nitrapyrin was combined with ammonium-acetate compared to a single application of ammonium-acetate. The treatments did not influence the sulfur content of onion leaves, but increased the bulb sulfur content in all cases. The highest bulb sulfur content was measured in the treatment of ammonium nitrate (240 kg N/ha) without Nitrapyrin application and in the treatment of ammonium nitrate (120 kg N/ha) with Nitrapyrin application. The highest sulfur uptake by leaves and bulbs was observed in combined treatment of urea (240 kg N/ha) and Nitrapyrin. The treatments influenced the N/S ratio of leaves. The increased dose of all fertilizers slightly increased the ratio of N/S in

leaves and in bulbs compared to values of lower fertilizer dose. The type of fertilizers did not alter these values in leaves, but influenced the bulb N/S ratio. Among lower doses the highest N/S ratio of bulb was obtained when urea was applied, while the combination of nitrification inhibitor with urea decreased the bulb N/S ratio. On the basis of our results it can be concluded, that the highest effect on the yield parameters, and nitrogen and sulfur status of onion was measured in the treatment of urea + Nitrapyrin at a rate of 240 kg N/ha. The combined application of urea (240 kg N/ha dose) with Nitrapyrin caused the highest plant biomass and nitrogen, sulfur uptake by onion. Nitrapyrin decreased the nitrification process of urea so plant could use the nitrogen more effectively.

Demonstrating the importance of sulfur in fertilizer plans for corn and barley using polyhalite

Timothy D. Lewis, Kiran Pavuluri and Robert Meakin
Sirius Minerals, 7-10 Manor Court, Manor Garth, Scarborough, YO11 3TU, U.K. (E-mail: timothy.lewis@siriusminerals.com)

Sulfur (S) can be considered the fourth essential plant nutrient in a fertilizer plan required by crops to reach their full potential in terms of yield and quality. Over the last century, S was supplied to soils and crops by anthropogenic emissions leading to atmospheric deposition of S. During the 1970s, SO_2 emissions peaked across Europe and North America and have been declining until the present day (Smith et al. 2011). Deficiency in soil S has started to manifest in areas of low deposition, such as the US and Europe, to the point where S is required in NPK fertilizer plans (Webb et al. 2016).

Polyhalite, commercially known as POLY4, compromises of potassium (14% K_2O), magnesium (6% MgO), calcium (17% CaO) and sulfur (48% SO_3) with the chemical formula $K_2SO_4{\cdot}MgSO_4{\cdot}2CaSO_4{\cdot}2H_2O$. The natural optimized ratios of nutrients can be useful for improving fertilizer use efficiency by addition of polyhalite to fertilizer plans. Exploration by Sirius Minerals and characterization work by Kemp et al. (2016) identified a resource of over 2.5 billion tonnes of polyhalite in the UK with an estimated supply for over 50 years. Therefore, research has been undertaken across the UK and North America to demonstrate the value of polyhalite as a multi-nutrient to supply K, Mg, Ca and S.

Trials on corn and barley were established in the US and the UK between 2014 and 2016 to determine the effectiveness of polyhalite under field conditions. The addition of polyhalite to NPK fertilizer plans to supply S demonstrated improvements in crop yield (15 – 135%), nutrient uptakes and plant quality compared to commercially available NPK plans. The use of polyhalite as a multi-nutrient fertilizer to supply K, Mg, Ca and S in fertilizer plans supports improvements in fertilizer efficiency.

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The environmental and agronomic impacts of γ-PGA on agricultural soils

Jie Li, Lei Zhang[1,2,3], Xueming Yang[3], Decai Gao[1,2], Lingli Wang[1], Zhanbo Wei[1] and Yuanliang Shi[1,]

[1]Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wenhua Road, Shenhe District, Shenyang, Liaoning 110016, China (E-mail: jieli@iae.ac.cn); [2]University of Chinese Academy of Sciences, 19 Yuquan Road, Shijingshan, Beijing 100049, China; [3]Harrow Research and Development Centre, Agriculture and Agri-Food Canada, 2585 County Road 20, Harrow, Ontario N0R 1G0, Canada

To demonstrate the responses of plant (Pakchoi) and soil to poly-γ-glutamic acid (γ-PGA) is essential to better understand the pathways of the promotional effect of γ-PGA on plant growth. In this study, the effects of γ-PGA on soil nutrient availability, plant nutrient uptake ability, plant metabolism and its distribution in a plant-soil system were tested using labeled γ-PGA synthesized from $^{13}C_1$-^{15}N-L-glutamic acid (L-Glu). γ-PGA significantly improved plant uptake of nitrogen (N), phosphorus (P), and potassium (K) and hence increased plant biomass. γ-PGA greatly strengthened the plant nutrient uptake capacity through enhancing both root biomass and activity. γ-PGA affected carbon (C) and N metabolism in plant, which was evidenced with increased soluble sugar contents and decreased nitrate and free amino acids contents. About 26.5% of the γ-PGA-N uptake during the first 24 h, after γ-PGA application, was in the form of intact organic molecular. At plant harvest, 29.7% and 59.4% of γ-PGA-^{15}N was recovered in plant and soil, respectively, with a 5.64% of plant N nutrition being derived from γ-PGA-N. The improved plant nutrient uptake capacity and soil nutrient availability by γ-PGA may partly explain the promotional effect of γ-PGA, however, the underlying reason may be closely related to L-Glu.

Spectral responses of sulfur deficiency in oilseed rape - first results of a field experiment

Holger Lilienthal, Heike Gerighausen, J. Krieger and Ewald Schnug
Institute for Crop and Soil Science, Julius Kühn-Institut (JKI), Braunschweig, Germany (E-mail: holger.lilienthal@julius-kuehn.de)

Sulfur deficiency in oilseed rape is clearly visible during flowering due to distinct color differences. Whilst sulfur deficiency is characterized by lightened white flowers, plants with good sulfur supply have strong yellow flowers. Remote sensing can be a tool to detect sulfur deficiency at flowering, especially with the advent of the *European Copernicus Programme* and its Sentinel-2 high resolution satellites. The imagery repeat frequency is five days at equator and even 2-3 days at higher latitudes, like in Germany. Remote sensing of oilseed rape at flowering must take place at the full bloom of the plants, since early or late flowering phases can falsify the result. Since this time span cannot always be covered by satellite remote sensing at regional scale, the spectral response of the plants under sulfur deficiency at other phenological stages needs to be investigated. A field trial has been performed to identify the spectral behavior of oilseed rape under different fertilizing conditions und phenological stages. Ten different genotypes and two different nitrogen (200 and 100 kg/ha), as well as two sulfur levels (40 and 10 kg/ha) have been applied. The plots were spectrally measured during the vegetation period to determine possible spectral indications for the detection of sulfur deficiency in oilseed rape next to the time of flowering.

Isotope studies in rock phosphates

Miyuki Maekawa[1], Roland Bol[2], Yajie Sun[2], Liankai Zhang[1], Silvia Haneklaus[1] and Ewald Schnug[1]
[1]Institute for Crop and Soil Science, Julius Kühn-Institut, Federal Research Institute for Cultivated Plants, Bundesallee 50, Braunschweig, Germany (E-mail: miyuki.maekawa@julius-kuehn.de); [2]Forschungszentrum Jülich IBG-3, Wilhelm-Johnen-Straße, 52428 Jülich, Germany

Isotope studies comprise tracer experiments and the determination of isotope ratios in fertilizers. So, it is for instance possible to discriminate crops in relation to soil characteristics and fertilizer type which have been labeled with the ^{34}S isotope by calculating δ^{34} S after measuring the ratio of stable $^{34}S/^{32}S$ isotope masses. The determination of the $^{87}Sr/^{86}Sr$ ratio proved to be suitable to distinguish phosphates bound in phosphorites from those in carbonatites. Such discrimination delivers further information about the contamination with heavy metals or rare earth elements which can be beneficiated and taken as raw material for industrial purposes. A major obstacle in fertilizer research is that the effective, long-term utilization of phosphate cannot be determined empirically as P is anisotopic with only one stabile isotope, ^{31}P so that fractionation studies are not feasible. Radioactive P tracers cannot be employed either as the half-live time of ^{33}P is with only 25.3 days and that of ^{32}P 14.3 days too short for long-term experiments. Accordingly, the geological origin of rock phosphates cannot be determined by stable P isotope ratios. Here, the δ^{18} O and activity ratio of $^{234}U/^{238}U$ are used to monitor provenances. It is the objective of this study to outline the current status of isotopic studies in rock phosphates, to summarize the significance of these data and to depict future analytical options in order to enable a proper attribution of the origin of rock phosphates and to follow up fluxes of contaminants in the environment.

Evaluation of polyhalite in soybean, maize and wheat in Pampean soils of Argentina

Ricardo J. Melgar[1], Luis Ventimiglia[1], Enrique Figueroa[2] and Fabio Vale[3]
[1]Instituto Nacional de Tecnologia Agropecuaria, Experimental Station Pergamino, Ruta 32 km 4.5, Pergamino 2700, Argentina (E-mail: rjrmelgar@gmail.com); [2]Experimental Station Mercedes, Argentina; [3]International Potash Institute (IPI), Latin American Coordinator, Piracicaba, Sao Paulo, Brazil

After long time being recognized as a major macronutrient in South American grain production, the supply of sulfur (S) in fertilizer programs does not represent a big challenge for farmers. Single superphosphate (12 %S), or gypsum (17 %S) are commonly used to fertilize major grain in Argentina and Brazil, either as single applications or in blends with more concentrated phosphate products. On extensive and widespread crop production areas like Pampean Region of Argentina, the need for more balanced S fertilizers with potassium and magnesium contents is of most importance to minimize the impact of extraction of these nutrients and maintain a more equilibrated balance. Fertilizer practices do not include K or Mg among the regular programs for extensive crops, like soybean, maize and wheat, by far the most important grains produced in Pampean region of Argentina. However, several decades of ever increasing unitary yields fertilized with N, P and lately S, has been clearly demonstrated the exploitation and land depletion of cationic nutrients when compared with pristine situations. Soil reserves are still above sufficiency levels, however, for K and Mg, but some soil types in some locations has been started to show some responses, although not statistically significant or economically appealing. On the other hand, sulfur is part of the regular program as well as P and N for cereals. Polyhalite is one of a number of evaporate minerals containing potassium. The content of impurities is low, and is almost entirely sodium chloride at a maximum inclusion of 5%. Polyhalite (dehydrate) is a single crystal complex with 2 molecules of water of crystallization. It is not a mixture of salts. The chemical formula is: $K_2Ca_2Mg(SO_4)_4{\cdot}2(H_2O)$. The main agronomic advantage is to have soluble forms of sulfur, as sulfate (19% of S) plus an additional natural content of other macronutrients: Potassium (14 % as K_2O), magnesium (6% as MgO), and calcium (17% CaO). The objective of this study is to evaluate the direct effect of Polyhalite on the grain yield of soybean, maize and wheat and the residual effect on the following crops and compare it with other fertilizing options to supply S.

Four field trials were conducted in Mollisols of Pampean region of Argentina in 2016-2017 season, one in Nueve de Julio with wheat and maize, and another in Mercedes with soybean and maize. All are being followed to evaluate the fertilizer residual effect on the succeeding crop. The experiment compared six treatments based on MAP plus different sources of sulfur in order to apply at sowing a single rate of P and S with several fertilizer combinations. (1) A control of regular MAP with no S applied; (2) single superphosphate (SSP, 0-20-0-12S), which supply P and S but no K nor Mg; (3) a bulk blend of MAP (0-52-0-

0S) with gypsum (0-0-0-17S); (4, 5 and 6) as Polyhalite, that was evaluated at three rates (100, 200 and 300 kg/ha) as bulk blends with MAP at different proportions, which supplied increasing amounts of K and Mg, but all the same N and P_2O_5. All fertilizer treatments were applied at sowing along the seed line with a planter. The maize and wheat received a banded fertilization with N as urea or UAN at V-6 and at tillering stage of each crop respectively. All crops were conducted with proper weed, pest and disease control according to normal practice of the area.

Significant increases in grain yields in response to sulfur (S) fertilization were found for the four grain crops, cultivated on soils low in available sulfur (S-SO_4) in the two locations. However, the contribution of K and Mg to give yields above the S applied treatments varied among the site and crops. No differences were found among S sources in Nueve de Julio for either wheat, or maize. On the other hand, in Mercedes, there were statistical differences for both maize and soybean among the polihalite and the other S sources treatments. The average response to sulfur was 19 %, but the contribution of Polyhalite over the other treatments that received S was about 7 %.

Treatment	Mercedes				Nueve de Julio			
	Maize		Soybean		Wheat		Maize	
	kg/ha	Rel. Yield	kg/ha	Rel. Yield	kg/ha	Rel. Yield	kg/ha	Rel. Yield
Control - No S	5356c	76%	1917d	62%	4719a	74%	13531a	87%
Single Superphosphate	5878bc	83%	2146dc	69%	5934b	94%	15222b	98%
MAP + Gypsum	7060a	100%	2406c	78%	6165b	97%	15183b	98%
MAP + Polyhalite 37/63	7029a	100%	2719b	88%	5919b	93%	15546b	100%
MAP + Polyhalite 22/78	6910a	98%	2993ba	97%	6089b	96%	15484b	100%
MAP + Polyhalite 16/84	6417ab	91%	3089a	100%	6345b	100%	15328b	99%
$p>F_{Treatment}$	0,007		<0,001		<0,001		<0,001	
$DLS_{5\%}$	945		296		474		706	
CV %	9,7		7,7		5,4		2,6	

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Interlaboratory comparison tests as a tool for quality improvement in fertilizer analysis methods and laboratory performance

H.-W. Olfs[1], J. Breuer[2], B. Dittrich[3], H. Hartwig[2], G. Kießling[4], R. Neuenfeldt[3], I. Paradies-Severin[5], K. Severin[6], W. Übelhör[2] and M. Schraml[7]

[1]Hochschule Osnabrück, Am Krümpel 31, D-49090 Osnabrück, Germany (E-mail: h-w.olfs@hs-osnabrueck.de); [2]LTZ Augustenberg, Neßlerstraße 25, D-76227 Karlsruhe, Germany; [3]Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Waldheimer Straße 219, D-01683 Nossen, Germany; [4]Thüringer Landesanstalt für Landwirtschaft, Naumburger Straße 98, D-07743 Jena, Germany; [5]LUFA Nord-West, Finkenborner Weg 1a, D-31787 Hameln, Germany; [6]Landwirtschaftskammer Niedersachsen, Johannssenstraße 2, D-30159 Hannover, Germany; [7]VDLUFA e.V., Obere Langgasse 40, D-67346 Speyer, Germany

In 2008, a yearly European wide interlaboratory comparison (ILC) program for fertilizers ("VDLUFA EU Fertilizer Ring Test") was established by the Association of German Agricultural Analytic and Research Institutes (VDLUFA) via its section 'fertilizer analysis'. The aims of this program are to (1) continuously review the reproducibility of lab test results, (2) to ensure quality assurance for laboratories offering fertilizer analysis and (3) to validate and (if proven necessary) revise analytical methods.

This ILC specially addresses laboratories notified by the EU Commission for fertilizer analysis, but is open for all laboratories offering fertilizer analysis worldwide. In the past years, up to 48 laboratories from 21 different countries participated. Based on EU fertilizer regulation methods (EC No 2003/2003) or other standardized methods (CEN, ISO) different analytes have to be determined depending on the fertilizer type. The statistical evaluation of the results from the participating labs is carried out using the software package 'ProLab' (QuoData GmbH, Dresden, Germany). Based on IUPAC guidelines, a robust method is applied (DIN 38402 A45, Q-method, Hampel estimate) and Zu-scores (tolerance limit |Zu| = 2.0) are calculated as a bias estimate to enable participating laboratories to evaluate their analytical performance.

In the recent ILC conducted in 2016, a magnesium calcium carbonate ("dolomitic limestone") fertilizer was sent to the labs and 13 different analytes had to be determined. Comparing the results for total Mg content for the participating laboratories reveals that equal Mg concentrations were detected using the official EU-methods based on complexometry or flame AAS (overall mean 10.87 % Mg) compared to an ICP-OES based method (overall mean 10.75 % Mg) with standard deviations of reproducibility of 0.37 (EU method) and 0.48 (ICP-OES). Based on these results that prove a successful validation of the ICP-OES method, which is an established procedure for Mg analysis in many laboratories all over Europe, a discussion on establishing this analytical procedure as new international standard can be initiated.

Suggestions for modifications of the analytical procedures resulting from this ILC are directly communicated to and discussed with German (DIN), European (CEN) and international standardization organizations (ISO). Furthermore, fertilizer material from this program is offered as ‘non-certified but validated’ reference fertilizer material for daily lab quality assuring routines. Due to upcoming changes in EU regulations, organic and organo-mineral fertilizers will be included in forthcoming ILC organized by VDLUFA.

Nitrous oxide emissions after slurry injection in maize cropping

H.-W. Olfs, M. Westerschulte, C.-P. Federolf, T. Zurheide, M.E. Vergara Hernandez, N. Neddermann, H. Pralle and D. Trautz
University of Applied Sciences Osnabrück, Faculty of Agricultural Sciences and Landscape Architecture, Am Krümpel 31, D-49090 Osnabrück, Germany (E-mail: h-w.olfs@hs-osnabrueck.de)

Agriculture in north-west Germany is characterized by intensive livestock farming and biogas production resulting in high amounts of organic manure and an increasing acreage of maize. To ensure proper early growth of maize most farmers use a side dress mineral nitrogen (N) and phosphorous (P) “starter fertilizer” in addition to broadcast slurry application. This regularly leads to nutrient surpluses at field level, which are at risk to be lost into non-agricultural ecosystems. Slurry injection below the maize seeds has been proven as an option to replace mineral NP starter fertilizer without impairing maize yields and quality. However, the highly concentrated slurry band in the soil might lead to favorable conditions for denitrification resulting in increased nitrous oxide (N_2O) emissions.

To compare different slurry application techniques with regard to N_2O emissions a maize field trial was conducted close to Osnabrück, Lower Saxony, Germany (52°20’ N, 07°58’ E; soil-type Gleyic Podzol; loamy sand) using a randomized complete block design (4 replications; plot size 3 m x 25 m). The slurry was applied with a four-row slurry injector at a spacing of 75 cm. The following treatments were conducted: (1) Control (no fertilization), (2) broadcast (slurry application by trailing hose applicator followed by immediate incorporation plus a side-banded 23N/10P starter fertilizer at planting, (3) injection (slurry injection), and (4) injection + NI (slurry injection with addition of the nitrification inhibitor ENTEC FL [EuroChem Agro GmbH, Mannheim, Germany] at a rate of 10 l ha^{-1}). Maize seeding was done 8 days later (seeding depth 4.5 cm) directly above the slurry bands.

For N_2O flux measurements PVC collars (78 cm x 78 cm, 15 cm in height) were installed in each plot centered above a maize row. Between April 2015 and March 2016 in total 54 gas samplings were conducted using white-colored PVC chambers (51 cm height). Gas analysis was done using a gas chromatograph equipped with an electron capture detector.

For the control treatment rather low N_2O emissions (> 25 µg N_2O-N/m²/h) were measured throughout the one-year measuring period. An increase in N_2O emissions occurred for the broadcast treatment shortly after slurry application resulting in slightly higher N_2O emissions compared to the control for the following 10 weeks. Injection of slurry into the soil resulted in a considerable intensification of N_2O release with a peak of more than 872 µg N_2O-N/m²/h end of May. Mixing the nitrification inhibitor ENTEC FL into the slurry prior to injection led to significantly lower soil nitrate concentrations for about 8 weeks until 6-leaf stage of maize reducing N_2O emissions by more than 50 % (maximum flux 374 µg N_2O-N/m²/h). From mid-July onwards until harvest of maize N_2O emissions were rather low for all treatments. This is most probably due to low soil nitrate concentrations resulting from N uptake by the maize plants. Also during the winter months until March 2016 very low N_2O emissions occurred and no relevant differences between the control and the slurry treatments could be detected. It can be concluded that the addition of a nitrification inhibitor to the slurry is an appropriate measure to reduce N_2O losses from injected slurry.

Cereal crop productivity in crop rotation under different management intensity

Virmantas Povilaitis, Sigitas Lazauskas and Šarūnas Antanaitis
Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto al. 1, Akademija, Kedainiai distr. LT-58344, Lithuania (E-mail: virmantas@lzi.lt)

Valinava long-term experiment (55.22° N, 23.51° E) established in 1991 at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry and is managed by the staff of the Department of Plant Nutrition and Agroecology. The experiment is situated on the terrace of the Dotnuvele River and occupies 4.4 ha. Prevailing soil is sandy loam and light loam *Endocalcari – Endohypogleyic Cambisol* (*CMg-p-w-can*). Carbonates depth is 40-60 cm. Soil pH_{KCl}: 7.2, total N content: 0.18 %; available phosphorus (P_{AL}): 66 mg kg^{-1}, available potassium (K_{AL}): 99 mg kg^{-1}. Crops are grown in 4-course crop rotation: spring barley (*Hordeum vulgare* L.), red clover (*Trifolium pratense, L.)*, winter wheat (*Triticum aestivum, L.*) and spring oilseed rape (*Brassica napus, L.*). Crops are grown under three levels of management intensity: a) conventional, b) integrated and c) organic, each replicated twice in space, occupies approximately 786 m^2 (32.2 m long, and 24.4 wide) and contains 6 sub-plots of 44 m^2 (20 m long, 2.2 m wide). Cereals in conventional and integrated agro-ecosystems were applied with herbicides, fungicides and insecticides and in organic system were grown without application of industrial fertilizers and plant protection measures. In conventional system, winter wheat grown for a target yield of 6 – 7 t ha^{-1}, and spring barley for 5 t ha^{-1}.

In this experiment measuring of drainage water runoff amount, ground water table levels, and crop yield and biomass production are performed. For soil moisture monitoring sensors, irrometers 'Watermarkt' are used. Experimental area includes plots with perennial grasses thus providing possibility to observe the changes in plant species. For indication of nitrogen and water deficit in cereal crops simulation with model DSSAT v4.0.2.0 is performed. These studies showed that water stress simulated by the DSSAT v4.0.2.0 model correlated relatively well with actual readings of irrometers. The correlation coefficient of three years' data in spring barley was 0.85, $p<0.05$, and in winter wheat – $r = 0.80$, $p<0.05$. The growing period of crops during the experimental years was warmer than the climate normal, with contrasting rainfall. Drainage water runoff measurements show that substantial part of precipitation was lost during the non-growth period resulting in lower levels of ground water table and temporary moisture deficiency in crops. Although soil moisture measurements and simulation indicated water stress in late spring or early summer in all experimental years, significant yield losses occurred only in a few cases. On average, the yield of winter wheat grown without fertilizers and pesticides was 67%, spring barley 70%, spring rape 47% and red clover 124% of that under conventional management.

Impact of sulfur nutrition and H_2S exposure on expression and activity of Group 1 sulfate transporters in developing *Brassica pekinensis* seedlings

Dharmendra H. Prajapati, Tahereh Aghajanzadeh and Luit J. De Kok
Laboratory of Plant Physiology, Groningen Institute for Evolutionary Life Sciences University of Groningen, P.O. Box 11103, 9700 CC Groningen, The Netherlands (E-mail: d.h.prajapati@rug.nl)

Sulfur is an essential nutrient for plants and is taken up as sulfate by the root. The uptake of sulfate by the root is under strict metabolic control and is presumably driven by the plant's sulfur demand for growth. In addition to sulfate taken up by the root plants are able to utilize foliarly absorbed H_2S as sulfur source for growth, resulting in a decreased sink capacity of the shoot for sulfur supplied by the root. Distinct sulfate transporters are involved in the uptake and distribution of sulfate in plants. The Group 1 sulfate transporters are responsible for the primary uptake of sulfate by the root. At an ample sulfate supply, Sultr1;2 appears to be responsible for the primary uptake of sulfate by roots of Brassicaceae, but upon sulfate deprivation also Sultr1;1 is expressed. The interaction between atmospheric H_2S nutrition and pedospheric sulfate nutrition and the sulfate deprivation on the expression and activity of the sulfate transporters Sultr1;1 and Sultr1;2 was studied developing *Brassica pekinensis* seedlings.

After germination, there was a gradual increase in the level of expression of Sultr1;2 in sulfate-sufficient roots, whereas expression of Sultr1;1 was hardly detectable (determined by qRT-PCR). Upon sulfate-deprivation there was a rapid and a substantial increase in expression of Sultr1;2 within one day, whereas the expression of Sulftr1;1 started to increase only after 2 days of deprivation. The increase in expression of the Group 1 transporters in sulfate-deprived developing seedling was accompanied by a substantial increase in the sulfate uptake capacity (up to 6-fold). Exposure of seedlings to atmospheric H_2S resulted in a concentration dependent decrease in the sulfate uptake capacity of both sulfate-sufficient and sulfate-deprived roots. However, H_2S exposure hardly affected the expression of both Sultr1;1 and Sultr1;2. The latter showed the absence of direct relation between the expression and the activity of the Group 1 sulfate transporters in roots of developing *B. pekinensis* seedlings. Moreover, there was no direct relation between the sulfate and water-soluble non-protein thiols content and the activity of the sulfate transporters in the root.

Sulfur availability from organic materials applied to winter wheat and winter oilseed rape crops

E. Sagoo[1], D. Munro[1], K. Smith[2], S.P. McGrath[3] and P. Berry[4]
[1]ADAS Boxworth, Battlegate Road, Boxworth, Cambridge, CB23 4NN, U.K. (E-mail: lizzie.sagoo@adas.co.uk); [2]ADAS Wolverhampton, U.K.; [3]Rothamsted Research, Harpenden, U.K.; [4]ADAS High Mowthorpe, U.K.

This paper reports results from experiments investigating crop available sulfur (S) supply from organic materials. The findings will help improve current recommendations on the use of organic materials as sources of crop available S and enable farmers to reduce their manufactured fertilizer S use accordingly. Field experiments were carried out at three sites cropped with winter wheat over three harvest years from 2010 to 2012 (two harvest years at each site; six harvest years in total). At each site, there were seven organic material treatments, namely autumn applied cattle farm yard manure (FYM), pig FYM, two biosolids products and broiler litter, and spring applied broiler litter and cattle or pig slurry. Crop yields and quality on the organic material treatments were compared with those on inorganic fertilizer S response treatments (supplying 0, 12.5, 25, 50 and 75 kg/ha SO_3) to determine the fertilizer S replacement values and hence the S availability of the applied organic materials.

Field experiments were carried out at four sites cropped with winter oilseed rape over three harvest years from 2014 to 2016 (two sites in 2014, one site in 2015 and one site in 2016). In 2014 and 2015 there were ten organic material treatments, namely autumn applied pig FYM, broiler litter and four biosolids products and spring applied cattle slurry, broiler litter and two biosolids products. In 2016 there were six organic material treatments including autumn applied cattle FYM, pig FYM, cattle slurry and pig slurry, and spring applied cattle slurry and pig slurry. Crop yields and quality on the organic material treatments were compared with those on inorganic fertilizer S response treatments (supplying 0, 30, 60, 90, 120 and 150 kg/ha SO_3) to determine the fertilizer S replacement values and hence the S availability of the applied organic materials.

There was a response to S at three of the six sites/years for the winter wheat and at all four winter oilseed rape sites. For the spring applied organic materials to winter wheat, ‘extractable’ SO_3 (i.e. readily available SO_3) was a good indicator of crop available S, ranging from *c.*15% of total SO_3 for cattle FYM to *c.*60% of total SO_3 for broiler litter. Results showed that for spring applied organic materials, ‘extractable’ SO_3 was equivalent to inorganic fertiliser S i.e. the S use efficiency for spring applications was 15% of total SO_3 for cattle FYM, 25% for pig FYM, 60% for broiler litter, 35% for slurry and 20% for biosolids. Lower S use efficiencies were measured from the autumn applied organic materials to winter wheat i.e. 5–10% of total SO_3 for livestock manures and 10–20% of total SO_3 for biosolids, suggesting that readily available S supplied by the organic materials was lost via overwinter leaching. In contrast, there was little effect of organic material application timing (autumn or spring) on S use efficiency by the oilseed rape crop. The increased S use efficiency from autumn applications to oilseed rape probably reflect greater crop S uptake by the oilseed rape crop in the period between application and the start of over winter drainage, resulting in lower S leaching losses.

This work has led to a better understanding of the available S supply from organic materials, allowing guidance to be produced for farmers on the availability of S from applications of organic materials (AHDB, 2017). This is likely to improve farm profitability by reducing S applications to crops receiving applications of organic materials.

AHDB (2017) Nutrient Management Guide (RB209) Section 2 – Organic materials. Available from http://www.ahdb.org.uk/projects/RB209.aspx

Stabilized fertilizer new technology research and development in China

Yuanliang Shi, Jie Li, Lingli Wang and Xiaoyu Shi
Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wenhua Road, Shenhe District, Shenyang, Liaoning 110016, China (E-mail: shiyl@iae.ac.cn)

Fertilizers are contemporarily the biggest commodities consumed in agricultural production. China has become a leading country worldwide in fertilizer production and consumption. The high amount of fertilizers applied in agriculture caused a series of negative environmental impacts such as eutrophication of surface and underground water bodies because of a low fertilizer utilization rate and nutrient losses by leaching and gaseous emissions to the environment. The NO_3^- content in vegetables exceeds the safety limit in intensive production areas and greenhouse gas emissions of N_2O and NO increase. China raises investments and efforts on fertilizer research in order to avoid and mitigate these environmental issues. After years of persisted research and refinement, significant improvements have been achieved in the production of stabilized fertilizers which are meanwhile a leading force among new types of fertilizers.

Role of autophagy during sulfur limitation

Leszek Tarnowski, Milagros Collados Rodriguez, Jerzy Brzywczy, Marzena Sieńko, Anna Niemiro, Anna Wawrzyńska and Agnieszka Sirko
Institute of Biochemistry and Biophysics Polish Academy of Sciences, ul. Pawinskiego 5A, 02-106 Warsaw, Poland (E-mail: asirko@ibb.waw.pl)

Autophagy, an important cellular degradation system plays an important role in plant growth, development and response to environmental stresses. In stress conditions it protects plant cells from the oxidative stress by degrading oxidized proteins and it recovers valuable substances from the degraded cellular components. On the other hand selective degradation of certain cellular targets (such as transcription factors or other regulatory proteins) by the plant autophagy machinery might be an effective strategy of reprogramming the cellular metabolism during shortage of nutrients. The role of selective autophagy and the selective autophagy cargo receptors is quite well characterized in mammals. Plants also possess selective autophagy cargo receptors, of similar to mammalian proteins domain architecture, named, depending on the plant species, NBR1 (Arabidopsis) or Joka2 (tobacco, potato). Through binding to ATG8s proteins NBR1/Joka2 deliver cargo to autophagosomes. However, their other partners, such as upstream regulators and degradation targets are not yet sufficiently investigated. Therefore, one of the focus of our work is identification of the protein partners of NBR1/Joka2 in normal and sulfur-deficiency conditions.

Moreover, plants with increased level of selective cargo receptors have been obtained in our laboratory and characterized in various growth conditions. Phenotypic differences between the wild type plants and the lines with the changed level of NBR1/Joka2 were rather subtle in normal growth conditions, however suggested that NBR1/Joka2 might be involved in nutrients deficiency response. Comparison of the transcriptomic profiles in shoots (rosette leaves) and roots of the wild type and the NBR1 overexpressing Arabidopsis revealed that NBR1 might be involved in certain processes related to abiotic stress response, such as cold or heat acclimation, regulation of circadian rhythm, flowering and ribosome biogenesis. Interestingly, increased level of NBR1 protein seems to affect the expression of numerous transcription factors. Involvement of NBR1 in processes and stresses concluded from the transcriptomic analysis is currently under investigation.

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Regulation of grapevine sulfate transporters gene expression under abiotic stress

Sílvia Tavares[1,2], Luísa Carvalho[1] and Sara Amâncio[1]
[1]LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal (E-mail: samport@isa.ulisboa.pt); [2]Section for Plant and Soil Science, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Frederiksberg C, Denmark

Plants assimilate sulfur (S) mostly through the synthesis of cysteine (Cys) from sulfate (SO_4^{2-}) absorbed by the roots from the soil solution. Sulfate, an essential macronutrient, is an oxidized form of sulfur used in protein biosynthesis but also in the synthesis of a myriad of compounds among each the tripeptide glutathione (GSH) with a central role in abiotic stress tolerance/resistance to abiotic stress.

Specialized proteins, anion-type transporters, mediate the transfer of sulfate from the soil solution into the cytosol of root cells and throughout the plant. Two kinds of transporters mediate the uptake and the distribution of sulfate: high-affinity sulfate transporter (HAST) and low-affinity sulfate transporter (LAST). Plants have developed a complex and sophisticated network of sulfate transporters. In grapevine genome it was possible to identify gene sequences for 12 sulfate transporters genes which share with all plant sulfate transporters two conserved domains, sulfate transp (PF00916) domain and STAS domain (PF01740). Grapevine predicted sulfate transporters protein sequences were divided by phylogeny analysis into the same groups already described for *Arabidopsis thaliana* sulfate transporters. However, some differences could be identified between grapevine and Arabidopsis: only two protein sequences assigned to group one (G1, high affinity transporters) and a higher number of group three (G3) transporters. Furthermore, in some of the G3 sulfate transporters evident signs of gene duplication were observed and three of these transporters were identified in tandem on chromosome 11. Interestingly, in grapevine berries two of these genes showed a high expression level while the third was not expressed in none of tested mRNA from leaves, roots, berries and cell cultures, indicating that this isoform is probably only expressed under very specific condition.

VviATPS1 and VviSULTR3;1, both predicted to target the chloroplast were located in the same chromosome. *VviSULTR1;1* a HAST which responds to sulfate deficiency conditions by transcription activation was also identified in tandem with one G2 sulfate transporters (*VviSULTR2;2*) and, although they do not meet the gene duplication criteria, they apparently share regulation similarity since they are both predicted targets for miRNA395. Conversely to *VviSULTR1;1*, *VviSULTR2;2* transcript level was unresponsive to cell cultures sulfate deficiency and its expression was higher in grapevine leaves.

In Mediterranean climate conditions field grapevine plants are subjected to strong abiotic stress, namely water stress. A cross talk between water stress and sulfate metabolism has been established previously. In the follow up, the gene expression of individual sulfate transporters was addressed in leaves and berries of distinct grapevine genotypes to tentatively depict different responses to abiotic stress.

Development of a system for fertilization with sulfur, reduction of ammonia emission and leaching of nitrate from slurry application and experiences with 5 years use in Denmark

Morten Toft
BioCover A/S, Veerst skovvej 6, 6600 Vejen, Denmark (E-mail: mt@biocover.dk)

Initiated by BioCover A/S, a group of companies were assembled (Aarhus University, SEGES, Grundfos) and committed to a joint development project – SyreN - with support from the Danish ministry for development and innovation to develop a system for fertilization with sulfur, reduction of ammonia emission and leaching of nitrate from slurry during application to fields. The group defined and created a demand specification for using sulfuric acid together with a slurry tanker during application with the purpose of being able to adjust the sulfur and ammonium nutrient values in the slurry. Following a successful research evaluation, the system was jointly developed to a pilot scale system for tests and following used in commercial slurry application. The system was tested according to the VERA protocol for ammonia emission. The extensive emission tests concluded an effectiveness of up to 70% reduction of ammonia emissions using sulfuric acid for acidification of slurry during application. Average reduction at pH 6.4 of 49%. App. 60% of all commercial applications results in following the recommended sulfur fertilization rates from the Danish extension services together with application of slurry at pH 6.4. 5 years of certified yield tests in winter wheat have concluded an average increase in yield of 1.7 hkg/ha. A total of 20 % of all slurry in Denmark is now being treated with acidification prior- or during application, with 120 SyreN systems operative in Denmark, using 16.000 m3 of sulfuric acid, without any reports of accidents over 5 years. Yield tests have shown up to 1.9 ton increased yield where sulfur deficiencies could be identified. Denmark is now committed to 24% ammonia emission reduction by 2020, compared to average 6% in EU. From 2016, an EUIinterreg project – Baltic sea acidification project, are establishing 7 pilot projects in all Baltic sea states. Software has been developed to allow the user to quantify amounts of sulfuric acid to be used and the nutrient values in the combined application. It also identifies the economy in using the system to optimize the effectiveness of organic fertilizers. A successful demonstration of commercial scale system to reduce both ammonia emission through acidification and reduction in leaching of nitrate through converting ammonia into ammonium. In addition, Sulfur fertilization through slurry application eliminates necessity of using NS fertilizers in certain crops, reducing traffic in fields.

Fangueiro, D., Hjorth M. and Fabrizio G. (2015) Acidification of animal slurry – a review. J. Environ. Manage. 149: 46-56.

Influence of sulfate containing potassium and magnesium fertilization on the yield amount and some content parameters of strawberry (*Fragaria ananassa*)

Imre Vágó, Jakab Loch And János Kátai
University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Agricultural Chemistry and Soil Science, Böszörményi Str. 138, H-4032 Debrecen, Hungary (E-mail: vago@agr.unideb.hu)

Potassium plays a role in the plant’s water management, promotes the cell elongation, it takes part in the synthesis of all kinds of carbohydrates: sugar, starch, and cellulose. That is why the proper potassium supply is important in strawberry production, too. Strawberry supplied with potassium well, synthesizes more sugar, so the yield will be sweeter, deliver more energy to human body, and more attractive for the consumers. Nowadays in plant production mostly potassium chloride is used as potassium fertilizer. Some plants, especially horticultural berries, strawberry in particular, react sensitive to chloride ions, because higher chloride concentration hinders the translocation of different assimilates. Therefore the application of chloride-free fertilizers such as potassium sulfate might be reasonable. Besides potassium, magnesium plays also a relevant role in plant life. It is important to watch out on magnesium supply on soils that are treated with a higher dosage of potassium, because of the antagonism between different ions, high potassium content can deteriorate the magnesium utilization. To avoid this negative effect, the potassium sulfate fertilization can be combined with magnesium sulfate application.

To study the potassium and magnesium supply of soils were set up the field experiments in the north eastern region of Hungary, near to Újfehértó, in an acidic soil (pH-KCl = 4.4) with sandy texture. The ammonium lactate – acetic acid soluble nutrient content is the following: 129.6 mg kg^{-1} P_2O_5; 208.8 mg kg^{-1} K_2O, 1243 mg Ca and 67.3 mg Mg kg^{-1} soil. The net area of each plot was: 2.8 x 3.75 m = 10.5 m^2, the distance between rows was 0.75 m, and the plant to plant distance was 0.25 m, so these were 60 plants in each plot. We used the sort “Polana” in our experiment. To reach a higher statistical reliability we set up our experiment in 6 replications, in a randomized arrangement.

On the basis of the results and their statistical analysis it was stated that the most favorable conditions for the strawberry development were ensured by the treatment with Patentkali (Table 1), which is a fertilizer that contains a combination of potassium sulfate and magnesium sulfate (in the form of Kieserit, $MgSO_4 \cdot H_2O$). The positive effect was confirmed by the statistical analysis at P = 0.1% probability level. Nutrients applied in form of sulfate inhibited the uptake of chloride-ions. It is presumable, that the decrease in the chloride-ion concentration of plants contributed to the yield increment.

Table 1. The yield of strawberry and the vitamin-C-, fructose and chloride-ion-content of the berries

Treatments	Parameters			
	Total yield (kg plot^{-1})	Vitamin C (mg kg^{-1})	Fructose (g kg^{-1})	Chloride (mg kg^{-1})
Without K and Mg	17.2	556	42.4	698
120 kg K_2O ha^{-1} (KCl)	17.3	503	39.9	727
120 kg K_2O ha^{-1} K_2SO_4)	19.7	586	49.0	449
120 kg K_2O ha^{-1} (K_2SO_4) + 40 kg MgO ha^{-1} ($MgSO_4$)	20.6	495	44.5	401
$SD_{5\%}$	1.1	96	9.2	118
F	21.4***	n.s.	n.s.	16.4***

*** significant at P = 0.1% level; n.s. = not significant

In situ evaluation of microbial contribution to nitrogen cycling in soil

Xudong Zhang[1], Hongbo He[1,2], Xiao Liu[1,3] and Guoqing Hu[1,4]
[1]Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China (E-mail: xdzhang@iae.ac.cn); [2]National Field Observation and Research Station of Shenyang Agroecosystems, Shenyang 110016, China; [3]College of Land Resources and Surveying Engineering, Shandong Agriculture and Engineering University, Jinan 250100, China; [4]National Engineering Laboratory for Efficient Utilization of Soil and Fertilizer Resources, College of Resources and Environment, Shandong Agricultural University, Taian 271018, China

There is increasing concern about the sustainability of agro-ecosystems, especially for the degraded arable soil. The only way to sustain soil quality is balancing the loss of soil organic matter (SOM) by returning crop residues, which is transformed and become SOM eventually. In addition, the immobilization of residue fertilizer nitrogen (N) is very important for reducing the environmental risk of fertilizer N loss. However, how different soil microorganisms contribute the transformation of both plant residue and fertilizer N and how the substrate-derived SOM is stabilized is not clear. The linkage of long term microbial function in extraneous N transformation and stabilization might merely be explicit by probing into the time-integrated response and acclimation of soil microorganisms to substrate supply. Differentiating between the newly synthesized and the inherent portions of microbial residue biomarker such as amino sugars, by ^{15}N tracing techniques, can provide an integrated view of long-term microbial dynamics, as opposed to live biomass. Hence, the response of different microbial populations to C and N supply was investigated based on the dynamics of glucosamine (GluN) and muramic acid (MurN). Here, we firstly undertook laboratory incubation with a silt loam soil amended with C and N isotope labeled substrates. A multi-year field experiment was then conducted in a temperate agro-ecosystem using crossly ^{15}N-labeled fertilizer and maize stalk. The ^{13}C and ^{15}N enrichments in the target amino sugar were identified by gas chromatography/mass spectrometry and those in soil was measured by elemental analyzer-isotope ratio mass spectrometer. In the incubation microcosms, the dynamic transformations of extraneous C and N into amino sugars were compound-specific, indicating different contributions of heterogeneous microbial residues over time. The extraneous N was preferentially utilized by bacteria firstly stimulated by available C but then dominantly converted by fungus population and stabilized in fungal products in succession. In the field experiment, the microbial transformation of fertilizer N depends on carbon availability, especially for the initially formation of bacterial residue, while the immobilization of maize stalk N was dominantly attributed to fungus-functioned decomposition of recalcitrant components with less extent passing through mineralization. The carbon-controlled substrate utilization induced a shift from a bacterial to fungal dominant community and the similar succession dynamics of fertilizer and maize stalk N related microbial populations.

There was no preferential utilization of inorganic N over stalk N into amino sugars during both the incubation course and the field tracing experiment. On the contrary, the microbial utilization efficiency of maize stalk N was remarkably higher than fertilizer in the field except for the first-year decomposition. The effective maintenance of maize stalk N in soil may be an important mechanism for soil N accumulation and replenishment in conservation tillage systems.

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